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Institute of Hygiene and Occupational Medicine, University of Essen, Germany
[1] Institute of Experimental Pathology, Hannover Medical School, Germany

Significance of cell type specific formation and elimination of DNA-adducts in respiratory tissues of hamster and rat induced by alkylating chemical carcinogens*

F. SEILER, B. REHN, K. KAMINO[1], M. EMURO, and J. BRUCH

With 4 figures and 1 table

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Address for correspondence: Dr. F. SEILER, Institute of Hygiene and Occupational Medicine, University of Essen, Hufelandstraße 55, D-45122 Essen, Germany

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Introduction

Chemical carcinogens and various dusts have been identified as cancerogenic factors in the air we breath. Previous studies in different animal species have shown that both the species and the test system (e. g. application method) have a great influence on the tumourigenic potency of the tested substances. Comparision of DNA adduct formation and repair at the cellular level among the commonly-used animal models may help in understanding species-specific differences in tumour formation and also help in selection of the most suitable test systems for risk assessment in humans. In a first set of experiments, lung and tracheal tissues of Syrian hamster and Wistar rats were investigated for formation and repair of the DNA-adduct O⁶-ethyldeoxyguanosine (O⁶-EtdGuo) following pulse alkylation with the monofunctional alkylating agents ethylnitrosourea (ENU) and diethylnitrosamine (DEN). ENU is a well characterized direct acting experimental carcinogen, whereas the metabolically activated DEN (by p450-monooxygenases) is found in trace amounts in environment. O⁶-EtdGuo is repaired predominantly by the repair protein O⁶-alkylguanine-transferase (AGT) (PEGG, 1990). Hamster and rat exhibit characteristic differences in alkylating agent-induced tumours of the respiratory region. Hamster tumours ocurred in high frequencies predominantly in the trachea; rat tumours occur only in the lung (for review: SCHMÄHL et al. 1980). Formation and elimination of DNA-adducts are quantified by a recently developed single cell immunofluorescence assay (ICA) (SEILER et al. 1993).

Material and methods

Male Syrian golden hamsters and Wistar rats were used for this investigation. The animals received a single intraperitoneal injection of either diethylnitrosamine (DEN) or ethylnitrosourea (ENU) a dose of 100 mg/kg body weight. The animals were killed 0.5, 2, 6, 12, 24, 18 and 96 (101, rat) hours after exposure. Negative control animals were treated with 0.9 % NaCl solution. Frozen sections (8 µm) were made from the selected organs and transfered on microscopic slides previously coated with ovalbumin.

ICA: Immunofluorescence staining was performed according to the slightly modified ICA protocol (SEILER et al. 1993). Cell samples were fixed in methanol and rehydrated in 2SSC. After treatment with RNAse A (200 µg/ml 2SSC) and RNAse T1 (50 units/ml 2SSC) for 1 h at 37 °C, cells were washed in 0.14 M NaCl, and DNA was denatured by incubation with 70 mM NaOH/ 40 % EtOH in 0.14 M NaCl for 5 min at 0 °C. After washing with PBS/1 % BSA cells were preincubated with PBS/20 % BSA for 20 min. O⁶-EtdGuo specific monoclonal antibody EM 2–3 (EBERLE 1988) was diluted to 0.2 µg/ml PBS/1 % BSA and slides were treated for 16 h at 4 °C, followed by intensive washing with PBS. Mouse anti rat-IgG F(ab')-fragments conjugated with rhodamine isothiocyanate (TRITC) (Dianova, Hamburg, Germany), diluted to 2 µg/ml in PBS/1 % BSA were added for 45 min, 37 °C. After washing, nuclear DNA was counterstained with the DNA-dye DAP (4.6-Diamidino-2-phenylindole) for 10 min, and mounted in PBS/20 % Glycerol,

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